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L6 L5 and (position ( probe and surface adj enhanced adj raman adj scattering  
adspectroscopy and metallic surface and metallic coating ))

75 L6

L5 L4 and (position (jp-07227287-\$.did. ))

75 L5

L4 L3 and (position F64)

75 L4

L3 L2 and (position E222)

75 L3

L2 Tsien.in.

127 L2

L1 6194548.pn.

1 L1

END OF SEARCH HISTORY

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TTC GGC TAC GGC GTG CAG TGC TTC GCC CGC TAC CCC GAC CAC ATG AAG Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys 305 310 315	240
CAG CAG GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu 320 325 330	288
CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu 335 340 345 350	336
GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly 355 360 365	384
ATC GAC TTC AAG GAC GAC GGC AAC ATC CTG GGC CAC AAG CTG GAG TAC Ile Asp Phe Lys Asp Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr 370 375 380	432
AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn 385 390 395	480
GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser 400 405 410	528
GTG CAG CCC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC Val Gln Pro Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly 415 420 425 430	576
CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC TAC CAG TCC GCC CTG Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu 435 440 445	624
AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe 450 455 460	672
GTG ACC GCC GCC GGG ATC ACT CAC GGC ATG GAC GAG CTG TAC AAG TAA Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys * 465 470 475	720

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 239 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu  
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly  
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile  
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr  
50 55 60 64

Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys  
65 70 75 80

Gln Gln Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu  
85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu  
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly  
115 120 125

-continued

Ile	Asp	Phe	Lys	Asp	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr
130						135				140					
Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn
145				150					155					160	
Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser
			165					170						175	
Val	Gln	Pro	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly
	180						185					190			
Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Tyr	Gln	Ser	Ala	Leu
	195					200						205			
Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Glu	Phe	
	210					215					220	Leu			
Val	Thr	Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys	
225				230					235						

What is claimed is:

1. A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, and a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V or S65I, and wherein said functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein.

2. The protein of claim 1, wherein the amino acid sequence differs from SEQ ID NO:2 by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

3. The protein of claim 1, wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

4. The protein of claim 1, wherein the amino acid sequence further comprises a mutation at a position selected from the group consisting of Y145, N146, H148, M153, and V163.

5. The protein of claim 1, wherein the amino acid sequence further comprises a folding mutation selected from the group consisting of F64L, V68L and S72A.

6. The protein of any one of claims 1 to 5, which is a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

7. The protein of claim 1, wherein the amino acid sequence differs from SEQ ID NO:2 by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; or S65G/S72A/T203Y.

8. The protein of claim 1, wherein the amino acid sequence differs from SEQ ID NO:2 by no more than the substitutions S65G/S72A/K79R/T203Y.

9. The protein of claim 1, wherein the amino acid sequence differs from SEQ ID NO:2 by no more than the substitutions T203Y/S65G/V68L/S72A.

\* \* \* \* \*